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Award Number: W81XWH-06-1-0081

TITLE: Prostate Cancer and Pesticide Exposure in Diverse Populations in California's Central Valley

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REPORT DATE: December 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-12-2007	Annual	20 Nov 2006 – 19 Nov 2007

4. TITLE AND SUBTITLE

Prostate Cancer and Pesticide Exposure in Diverse Populations in California's Central Valley

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0081

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Southern California
Los Angeles, CA 90089-1147

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Not Provided

15. SUBJECT TERMS
Not Provided

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	18	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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In this second report, we outline progress since the inception of the award, noting using underline, additional progress since the last report.

In short, we have completed a total of 170 case and 204 control interviews, and have updated analyses (mid year, for the PCRP meeting in Atlanta) based on 136 cases and 137 controls, for whom data has been cleaned to date. We have now completed case and control recruitment.

To finish the study in the coming year, we will incorporate data from all 170 cases and 204 controls into analyses of pesticide exposure and prostate cancer, and complete our other analyses of the representativeness of the cases and controls.

A. INTRODUCTION:

There is some evidence that pesticide exposure is a risk factor for prostate cancer. Some pesticides, classified as endocrine-disrupting chemicals (EDCs), can affect normal hormone function. Variations in hormone levels affect prostate cancer risk, since normal growth of the prostate gland is dependent on a critical balance of androgen levels. Pesticides may affect hormone function by mimicking hormones, affecting enzyme systems involved in hormone metabolism, or directly affecting the brain regions involved in hormone functioning. A possible involvement of pesticides in prostate carcinogenesis is suggested by findings among farmers in studies of occupation and prostate cancer. The overall association reported by recent meta-analyses of farming and prostate cancer report a summary relative risk of 1.1, but the majority of studies with relatively large numbers of subjects consistently showed excess relative risks of prostate cancer ranging from 1.06 to 5.0. This limited evidence may well be inconclusive because of the difficulty in measuring true pesticide exposure – all these studies relied on self-reported occupational exposure, resulting in bias towards the null, and the omission of non-occupational environmental exposures (e.g. residences downwind of application sites). A large-scale population-based case-control study in California's Central Valley, the nation's leading user of pesticides, simultaneously assessing genetic and environmental risk factors for prostate cancer in an ethnically-diverse population with varying occupational and residential exposures to pesticides would go a long way to further refining knowledge of prostate cancer etiology. However, the complexities of such a study warrant excellent pilot data. We have been evaluating for some time now the use of Pesticide Use Reporting (PUR) data, refined by additional data on land use, in a Geographical Information System (GIS) to obtain objective historical pesticide exposure estimates.

This project is a pilot case-control study of pesticide exposure and prostate cancer, hypothesizing that (1) attenuation of estimates of the relative risk of pesticide exposure and prostate cancer in the absence of full (residential and occupational) historical pesticide exposures is significant, and could explain null

findings to date; (2) our proposed method of recruiting and approaching cases and controls to a large population-based case-control study will result in acceptable response rates, but our sample will be biased with respect to socioeconomic status, race, and disease characteristics – we will preferentially recruit higher SES, white males with localized disease; (3) We will be able to obtain sufficient DNA from mailed buccal swab kits to assess effect modification by known relevant genes, and have sufficient stored DNA to assess the impact of genes that may be discovered in future.

B. PROGRESS TOWARDS SPECIFIC AIMS.

Specific Aims outlined in the Statement of Work were:
1. show that **historical** residential and PUR/land use data provides substantial **reduction in exposure misclassification** in both prostate cancer cases and controls compared to estimates based only on **current** residential addresses and PUR/land use data information alone
2. demonstrate the **feasibility** of conducting a case-control study of biochemical and environmental risk factors (especially pesticide exposure), susceptibility genes, and their interactions for prostate cancer in the Central Valley. In particular, we will demonstrate the feasibility of our **case selection method**, **control selection method**, and **methods of obtaining buccal DNA** for genetic hypotheses.

Accomplishments to date:

1. Development of the GIS for determining exposure to pesticides.

The process for estimating pesticide exposure in this study relies on combining data from California's Pesticide Use Registry (PUR) and land use (PLSS) data to determine the exact location of applied pesticides.

We developed an automated program for combining the PUR and PLSS data within a GIS – this automated process was custom programmed in ArcGIS, and can be updated with new PUR and PLSS data as they become available. It also allows us to use any historical residential data (e.g. from other case-control studies) and generate pesticide exposure estimates.

We are currently using this GIS in this project to determine pesticide exposures, and in other studies where pesticide exposures are required (e.g. an ongoing study of risk factors for breast cancer in the inhabitants of California's Central Valley).

Year 2 accomplishments: Since last progress report we have further refined our computer model so that it can run on many thousands of observations (previously this was not possible because of limitations in ArcGIS – we removed those limitations by programming a separate interface in .Net). This is required so that we can generate pesticide analyses for thousands of population-based points (randomly selected tax assessor parcel locations) for comparison of exposure assessment misclassification in the cases and controls (Aim 2). This also means that the code can be more easily shared with other investigators who wish to use the approach.

2. Development of questionnaire

We developed, piloted and refined a questionnaire that ascertained prostate cancer risk factor information, as well as detailed historical residential data (to incorporate into the pesticide exposure assessment) and detailed information on in-home and occupational exposure to pesticides. This questionnaire has been used throughout the study, and will be available as a deliverable at the conclusion of the study. Now the questionnaire has been used among 374 individuals (170 cases and 204 controls), and we have noted changes required for clarity. These will be incorporated into the final deliverable.

In addition, we have developed a data entry system in SAS for the questionnaire that can also be made available upon request (there are many hundred of items in the questionnaire, and an accompanying database design will greatly reduce the effort of anyone wishing to use it).

3. Recruitment and interview of prostate cancer cases

Aim 2. was to demonstrate the feasibility of conducting a case-control study of biochemical and environmental risk factors (especially pesticide exposure), susceptibility genes, and their interactions for prostate cancer in the Central Valley. In particular, we wished to demonstrate the feasibility of our case selection method, and methods of obtaining buccal DNA for genetic hypotheses.

We estimated we would be able to obtain 60 cases and controls, and in fact have recruited and interviewed 170 cases, and 204 controls. We have completed case and control recruitment.

We analyzed the representativeness of the cases included in our study (the response rate, after removing those cases we had no contact information for, was 64% - which is high for this kind of study which did not use rapid case ascertainment – but tells us nothing of the probability that we included a biased sample of cases). The results are summarized in Table 1, which compares the cases we obtained from the population-based Central California Cancer Registry with the cases we were able to interview ('surveyed cases') and those finally included in the analysis above (those providing informed consent and saliva sample for DNA analyses).

[UPDATED TABLE FOLLOWS]
Table 1 Comparison of interviewed prostate cases with those selected from the population-based Cancer Registry.

Prostate Cancer, N (%)		Attempted cases		Analysis cases	
		NH white	Hispanic	NH white	Hispanic
Diagnosis Age, y	60-64	92 (27.46)	58 (25.78)	35 (28.23)	15 (32.61)
	65-69	130 (38.81)	84 (37.33)	47 (37.90)	18 (39.13)
	70-74	113 (33.73)	63 (36.89)	42 (33.87)	13 (28.26)
Stage	IN SITU			0 (0.00)	0 (0.00)
	LOCALIZED	277 (82.69)	180 (80.00)	104 (83.87)	38 (82.61)
	REGIONAL, DIRECT EXTENSIONS ONLY	38 (11.34)	29 (12.89)	14 (11.29)	4 (8.70)
	REGIONAL, NODES ONLY	4 (1.19)	0 (0.00)	2 (1.61)	0 (0.00)

	REGIONAL, DIRECT EXTENSION AND NODES	2 (0.60)	4 (1.78)	2 (1.61)	1 (2.17)
	DISTANT METASTASES OR SYSTEMIC DISEASE (REMOTE)	8 (2.39)	8 (3.56)	1 (0.81)	2 (4.35)
	UNSTAGEABLE; UNKNOWN	5 (1.49)	4 (1.78)	1 (0.81)	1 (2.17)
	MISSING	1 (0.30)	0 (0.00)	0 (0.00)	0 (0.00)
Birthplace	UNITED STATES	159 (47.46)	70 (31.11)	62 (50.00)	16 (34.78)
	OTHER	11 (3.28)	58 (25.78)	5 (4.03)	19 (41.30)
	MISSING	165 (49.25)	97 (43.11)	57 (45.97)	11 (23.91)
		335	225	124	46

While we expected that we would preferentially select cases with a lower stage disease and cases more likely to be younger, and with a birthplace in the U.S. (the former two affecting generalizablilty of general prostate cancer risk factor information, the latter affecting our lifetime estimates of pesticide exposure from residential history), we found instead that there were few, if any, differences between the population-based sample of cases, and those included in the final analysis. This leads us to conclude that our case-control method yields a relatively unbiased source of cases and controls for this study design. While the response rate in the Hispanic population was lower than among the non-Hispanic White population, this is a misleading figure because we only added Hispanic cases near the end of the study, and had less time to recruit them. The recruitment-time specific response rates were very similar in Hispanic and non-Hispanic white populations in this study.

Extracting DNA from saliva specimens

We used the Oragene saliva kit to obtain specimens from all participants. We mailed participants the kits, and they were returned to us by mail. Specimens were stored at room temperature for 1-3 weeks before being processed by the lab.

We quantified DNA yield from saliva specimens. The overall mean yield was 29,817 ng, with a minimum of 335 ng, a maximum of 227,441 ng. 58% of samples had greater than 20,000 ng.

4. Initial analyses of pesticide exposure and prostate cancer risk

We used our GIS-based Residential Ambient Pesticide Exposure Software (GRAPES GRAPES) tool to estimate lifetime and age-specific exposures to a variety of pesticides and herbicides using residential history information, and combined data from the California Pesticide Use Registry (PUR) and Land Use Information, both available for years from 1974 to 1999. We have previously described how the latter are combined to

produce an accurate estimate of year-specific pesticide application in small geographical areas (Ritz and Rull). Our GRAPES model combines PUR and LU data for each reported residence for the lifetime history of cases and controls.

We hypothesized that previous studies of prostate cancer and pesticide exposure that only considered exposures occurring at time of diagnosis would underestimate the true relationship due to (1) random misclassification (inaccurate estimation of exposure) resulting in bias towards the null (2) specifically underestimating exposure in cases only, resulting in a differential bias, but still with a net effect of bias towards the null.

In our initial analyses of these effects, we focused on the main pesticide groupings that have been shown to have relationships with prostate cancer, namely methyl bromide, captan, and simazine. Ongoing analyses are assessing other pesticides, and groupings of pesticides, such as organochlorines.

We calculated exposures for (1) diagnosis year only (2) life time (ie age 0 to age at diagnosis) – for this exposure, we assumed that year 1974 pesticide use continued back through time to the earliest year required (3) the period 1974 to 1999 only (the years for which PUR/LU data were available (4) accumulated exposures in the 10 years prior to diagnosis only (5) accumulated exposures in the 20 years prior to diagnosis only.

Mean exposure levels are summarized in Table 2.

[UPDATED TABLE BELOW]

Table 2 Estimated exposure levels for key pesticides in cases and controls in California's Central Valley, 2005 - 2006 (in pounds)

		Mean	Std Err	Max (*)	Count (†)
Methyl Bromide					
Case	*DX Year*	74.79	42.94	5359.55	130
	Life time	20.95	6.06	517.29	134
	1974 - 1999	33.44	8.84	582.98	120
	10 years prior to DX	11.09	3.17	198.36	120
	15 years prior to DX	6.15	1.97	165.92	113
Control	*DX Year*	19.30	10.30	963.58	122
	Life time	9.90	3.08	316.54	134
	1974 - 1999	18.03	6.54	706.12	132
	10 years prior to DX	6.33	2.91	352.42	129
	15 years prior to DX	5.76	3.34	385.12	119
Captan					
Case	*DX Year*	3.48	1.43	115.25	130
	Life time	1.29	0.31	26.94	134
	1974 - 1999	2.22	0.54	39.35	120
	10 years prior to DX	1.33	0.39	36.97	120
	15 years prior to DX	1.06	0.27	16.13	113
Control	*DX Year*	0.85	0.54	62.13	122
	Life time	0.72	0.32	40.57	134
	1974 - 1999	0.98	0.41	46.27	132
	10 years prior to DX	0.54	0.20	21.65	129
	15 years prior to DX	0.44	0.15	14.73	119
Simazine					

	DX Year	5.82	1.51	106.03	130
	Life time	1.92	0.36	20.83	134
Case	*1974 - 1999*	2.67	0.65	36.64	120
	10 years prior to DX	1.36	0.28	19.37	120
	15 years prior to DX	1.13	0.29	23.40	113
	DX Year	3.63	1.83	208.69	122
	Life time	1.65	0.44	37.69	134
Control	*1974 - 1999*	2.32	0.95	114.20	132
	10 years prior to DX	1.01	0.27	21.24	129
	15 years prior to DX	0.92	0.30	23.01	119
Octane Chloride Group					
	DX Year	2.53	1.61	203.43	130
	Life time	4.68	0.87	61.26	134
Case	*1974 - 1999*	4.35	0.89	70.18	120
	10 years prior to DX	5.90	1.13	71.46	120
	15 years prior to DX	6.22	1.29	78.88	113
	DX Year	0.38	0.17	12.68	122
	Life time	1.71	0.35	28.73	134
Control	*1974 - 1999*	1.29	0.39	42.97	132
	10 years prior to DX	2.16	0.45	33.15	129
	15 years prior to DX	2.32	0.54	36.05	119

* The minimum exposure is always 0, namely unexposed

† Number of the patients with observable exposure

We then calculated crude odds ratios (ORs) and ORs adjusted for age, race, and home pesticide use (yes/no for ever used pesticides in the home). These results are outlined in Tables 3a-d for each of the exposure time periods noted above, which also provide 95% CIs for effect estimates, and p-values for the difference between exposure levels. Because the distribution of exposure was skewed, we provide both an estimate of the relative risk for any exposure (ie >0), and for two levels of exposure (medium and high, depending on the distribution of exposure), both compared to 0 exposure as a baseline.

[UPDATED TABLE FOLLOWS]

Table 3a. Relative risk estimates for prostate cancer with exposure to Methyl Bromide in California's Central Valley 2005 - 2006

Methyl Bromide

Exposure Type	Frequency		Crude				Adjusted			
	Control	Case	OR	L95	U95	*p*-value	OR (*)	L95	U95	*p*-value
DX Year Exposure										
Missing	15	6	-	-	-		-	-	-	
Unexposed	114	108	1.00	-	-		1.00	-	-	
Exposed	8	22	2.90	1.24	6.80	0.01	**2.49**	1.05	5.94	0.04
Low (†)	3	3	1.06	0.21	5.34	0.03	1.08	0.21	5.70	0.08
High (†)	5	19	**4.01**	1.45	11.12		**3.31**	1.17	9.38	
Life Time										
Missing	3	2	-	-	-		-	-	-	
Unexposed	76	62	1.00	-	-		1.00	-	-	
Exposed	58	72	1.52	0.94	2.46	0.09	1.42	0.86	2.34	0.17
Low	27	33	1.50	0.81	2.76	0.23	1.49	0.80	2.80	0.38
High	31	39	**1.54**	0.86	2.75		1.36	0.74	2.48	
1974 - 1999										
Missing	5	16	-	-	-		-	-	-	
Unexposed	75	49	1.00	-	-		1.00	-	-	
Exposed	57	71	**1.91**	1.16	3.15	0.01	**1.82**	1.08	3.06	0.03
Low	24	32	**2.04**	1.08	3.87	0.04	**2.07**	1.07	4.01	0.07
High	33	39	**1.81**	1.01	3.25		1.63	0.89	3.01	
10 year prior DX										
Missing	8	16	-	-	-		-	-	-	
Unexposed	78	58	1.00	-	-		1.00	-	-	
Exposed	51	62	1.63	0.99	2.70	0.06	1.55	0.92	2.62	0.10
Low	33	34	1.39	0.77	2.49	0.09	1.42	0.78	2.60	0.22
High	18	28	2.09	1.06	4.14		1.80	0.87	3.71	
15 year prior DX										
Missing	18	23	-	-	-		-	-	-	
Unexposed	75	56	1.00	-	-		1.00	-	-	
Exposed	44	57	1.73	1.03	2.93	0.04	1.59	0.93	2.74	0.09
Low	28	38	1.82	1.00	3.31	0.11	1.71	0.93	3.17	0.22
High	16	19	1.59	0.75	3.37		1.38	0.63	3.02	

*: Adjusted for age, ethnicity and home pesticide use , significant results are highlighted

†: Low exposure: > 0 and < 3 pounds; High exposure: ≥ 3 pounds

Table 3b. Relative risk estimates for prostate cancer with exposure to Captan in California's Central Valley 2005 - 2006

Captan

Exposure Type	Frequency		Crude				Adjusted			
	Control	Case	OR	L95	U95	*p*-value	OR (*)	L95	U95	*p*-value
DX Year Exposure										
Missing	15	6	-	-	-		-	-	-	
Unexposed	115	114	1.00	-	-		1.00	-	-	
Exposed	7	16	2.30	0.91	5.81	0.08	2.24	0.88	5.72	0.09
Low (†)	0	2	-	-	-	0.35	-	-	-	0.39
High (†)	7	14	2.02	0.79	5.18		1.95	0.75	5.09	
Life Time										
Missing	3	2	-	-	-		-	-	-	
Unexposed	93	81	1.00	-	-		1.00	-	-	
Exposed	41	53	1.48	0.90	2.46	0.13	1.42	0.84	2.40	0.19
Low	21	16	0.87	0.43	1.79	0.04	0.83	0.40	1.72	0.05
High	20	37	**2.12**	1.14	3.95		**2.09**	1.09	3.99	
1974 - 1999										
Missing	5	16	-	-	-		-	-	-	
Unexposed	93	69	1.00	-	-		1.00	-	-	
Exposed	39	51	**1.76**	1.05	2.97	0.03	1.64	0.96	2.82	0.07
Low	17	11	0.87	0.38	1.98	0.01	0.82	0.35	1.89	0.02
High	22	40	**2.45**	1.34	4.49		**2.29**	1.23	4.28	
10 year prior DX										
Missing	8	16	-	-	-		-	-	-	
Unexposed	90	72	1.00	-	-		1.00	-	-	
Exposed	39	48	1.54	0.91	2.60	0.11	1.46	0.85	2.51	0.17
Low	19	14	0.92	0.43	1.96	0.05	0.89	0.41	1.93	0.09
High	20	34	**2.12**	1.13	4.00		**2.01**	1.04	3.88	
15 year prior DX										
Missing	18	23	-	-	-		-	-	-	
Unexposed	85	67	1.00	-	-		1.00	-	-	
Exposed	34	46	1.72	0.99	2.97	0.05	1.60	0.91	2.83	0.10
Low	16	13	1.03	0.46	2.29	0.04	1.00	0.44	2.27	0.08
High	18	33	**2.33**	1.21	4.49		**2.16**	1.08	4.30	

*: Adjusted for age, ethnicity and home pesticide use

†: Low exposure: > 0 and < 0.5 pounds; High exposure: ≥ 0.5 pounds

Table 3c. Relative risk estimates for prostate cancer with exposure to Simazine in California's Central Valley 2005 - 2006

Simazine

Exposure Type	Frequency		Crude				Adjusted			
	Control	Case	OR	L95	U95	*p*-value	OR (*)	L95	U95	*p*-value
DX Year Exposure										
Missing	15	6	-	-	-		-	-	-	
Unexposed	105	97	1.00	-	-		1.00	-	-	
Exposed	17	33	**2.10**	1.10	4.01	0.02	1.90	0.98	3.68	0.06
Low (†)	7	10	1.55	0.57	4.22	0.06	1.33	0.48	3.73	0.12
High (†)	10	23	**2.49**	1.13	5.49		**2.30**	1.03	5.14	
Life Time										
Missing	3	2	-	-	-		-	-	-	
Unexposed	72	67	1.00	-	-		1.00	-	-	
Exposed	62	67	1.16	0.72	1.88	0.54	1.08	0.66	1.79	0.75
Low	49	41	0.90	0.53	1.53	0.08	0.82	0.47	1.44	0.08
High	13	26	**2.15**	1.02	4.52		2.03	0.95	4.35	
1974 - 1999										
Missing	5	16	-	-	-		-	-	-	
Unexposed	77	64	1.00	-	-		1.00	-	-	
Exposed	55	56	1.22	0.74	2.02	0.42	1.11	0.66	1.86	0.69
Low	42	34	0.97	0.56	1.71	0.16	0.85	0.48	1.52	0.13
High	13	22	2.04	0.95	4.36		1.99	0.91	4.36	
10 year prior DX										
Missing	8	16	-	-	-		-	-	-	
Unexposed	72	63	1.00	-	-		1.00	-	-	
Exposed	57	57	1.14	0.69	1.88	0.60	1.09	0.65	1.84	0.74
Low	48	38	0.90	0.53	1.56	0.09	0.87	0.49	1.54	0.13
High	9	19	**2.41**	1.02	5.71		2.21	0.92	5.35	
15 year prior DX										
Missing	18	23	-	-	-		-	-	-	
Unexposed	71	62	1.00	-	-		1.00	-	-	
Exposed	48	51	1.22	0.72	2.05	0.46	1.19	0.69	2.05	0.54
Low	40	37	1.06	0.60	1.86	0.34	1.07	0.60	1.94	0.57
High	8	14	2.00	0.79	5.09		1.68	0.65	4.38	

*: Adjusted for age, ethnicity and home pesticide use, significant results are highlighted

†: Low exposure: > 0 and < 3 pounds; High exposure: ≥ 3 pounds

Table 3d. Relative risk estimates for prostate cancer with exposure to Organochlorine Group in California's Central Valley 2005 - 2006

Organochlorine Group

Exposure Type	Frequency		Crude				Adjusted			
	Control	Case	OR	L95	U95	*p*-value	OR(*)	L95	U95	*p*-value
DX Year Exposure										
Missing	15	6	-	-	-		-	-	-	
Unexposed	113	115	1.00	-	-		1.00	-	-	
Exposed	9	15	1.64	0.69	3.89	0.26	1.37	0.56	3.33	0.49
Low (†)	1	2	1.97	0.18	21.97	0.53	1.68	0.14	19.86	0.78
High (†)	8	13	1.60	0.64	4.00		1.33	0.52	3.41	
Life Time										
Missing	3	2	-	-	-		-	-	-	
Unexposed	55	45	1.00	-	-		1.00	-	-	
Exposed	79	89	1.38	0.84	2.26	0.21	1.29	0.77	2.16	0.32
Low	38	31	1.00	0.54	1.85	0.10	0.94	0.50	1.78	0.15
High	41	58	1.73	0.99	3.03		1.63	0.91	2.91	
1974 - 1999										
Missing	5	16	-	-	-		-	-	-	
Unexposed	70	42	1.00	-	-		1.00	-	-	
Exposed	62	78	**2.10**	1.26	3.48	0.00	**1.85**	1.09	3.11	0.02
Low	30	36	**2.00**	1.08	3.71	0.02	1.71	0.91	3.24	0.07
High	32	42	**2.19**	1.20	3.98		**1.97**	1.07	3.64	
10 year prior DX										
Missing	8	16	-	-	-		-	-	-	
Unexposed	54	35	1.00	-	-		1.00	-	-	
Exposed	75	85	**1.75**	1.03	2.96	0.04	1.70	0.99	2.93	0.05
Low	33	24	1.12	0.57	2.21	0.01	1.12	0.55	2.25	0.03
High	42	61	**2.24**	1.26	4.00		**2.15**	1.18	3.90	
15 year prior DX										
Missing	18	23	-	-	-		-	-	-	
Unexposed	53	35	1.00	-	-		1.00	-	-	
Exposed	66	78	**1.79**	1.04	3.07	0.03	**1.77**	1.01	3.08	0.05
Low	27	23	1.29	0.64	2.60	0.04	1.34	0.65	2.76	0.07
High	39	55	**2.14**	1.18	3.86		**2.07**	1.12	3.82	

*: Adjusted for age, ethnicity and home pesticide use, significant results are highlighted

†: Low exposure: > 0 and < 1 pounds; High exposure: ≥ 1 pounds

These results clearly show:

- Different estimates of relative risk are obtained when considering only diagnosis year exposures compared to exposures over time which includes life time, 1974 – 1999 and so on. However, these do not always result in a bias towards the null: the effect is pesticide-specific, which presumably is a result of the variation

in application of pesticides over time. Pesticides that were more commonly applied recently will be affected differently from those more commonly applied decades ago.

- There appear to be significant increased risks of prostate cancer associated with exposure to methyl bromide, captan, simazine and organochlorine group after adjusting to some common potential confounding factor including age, race and home pesticide usage (this is currently a yes/no variable, we are presently investigating the actual pesticides used in the home). The magnitudes of the risks represented by adjusted odds ratios vary according to different exposures and exposure evaluation methods, ranging from 1 to 4, with methyle bromide, captan and organochlorine all showing multiple significant ORs while simazine only showing significant risk for high diagnosis year exposure. We also noticed that all but the exposure to methyl bromide expressed an increasing trend in risk when comparing high level exposure to low level exposure. In fact, most low level exposure almost showed no increased risk while many high level exposures did. These results are in agreement with studies of occupational exposure to pesticides where exposure levels far exceed those to be expected in the residential environment, which we have measured here. These results must be heeded with caution because of they are subject to missing values and limited sample size.
-

Recruitment of an unbiased sample of control subjects by visiting residential tax assessor parcel units in the study area.

We recently initiated home visits to recruit control subjects, as outlined in the Statement of Work. To date we have made 8 field trips into the Central Valley, each consisting of 3 days work by 2 teams of 2 interviewers. Key characteristics of this effort are:

- We have visited 434 households from 1,093 eligible parcels. We also attempted to visit two additional neighbors for each parcel. To date we have visited 813 such neighbors neighbors.
- We have recruited 23 control subjects, who have been interviewed.
- We have developed software for a handheld computer (PDA) with a built in GPS device that also validates the location of residential parcels (for future validation of residential history in our GIS) – this PDA is also used as the primary data collection tool for enumerating households and collecting baseline eligibility data for controls.

None of these controls occur in the above data, because at the time of writing this annual report, their data has not been cleaned for analysis.

5. **Continuation into Year 3.**
    - We will continue recruiting control subjects with our home visit protocol, and compare the controls recruited using this method to those found by phone contact, hypothesizing that the home visit control subjects will be a more representative sample of the underlying population.
    - We will compare controls to the underlying population in two ways: (1) by comparing the control demographics (age, race, SES) to census data from the census tracts from which they were obtained; (2) by comparing the pesticide exposures in our GRAPES model for controls to the average values for all areas under study, to determine if the selected controls had differing pesticide exposures than the underlying population (resulting in biased exposure estimates). In both cases we will quantify the potential bias.
    - We will continue analyses of other pesticides and classes of pesticides.
    - We will adjust the final results for other critical risk factors and potential effect modifiers, such as prostate cancer stage, and home use of pesticides (the actual pesticides, which we are currently categorizing into the same groups as occur in GRAPES – we previously considered only self report of ever/never home pesticide use).

C. KEY RESEARCH ACCOMPLISHMENTS:

Despite the fact that we are still in the process of collecting data, results to date appear to clearly show:

- Different estimates of relative risk are obtained when considering only diagnosis year exposures compared to lifetime exposures. However, these do not always result in a bias towards the null: the effect is pesticide-specific, which presumably is a result of the variation in application of pesticides over time. Pesticides that were more commonly applied recently will be affected differently from those more commonly applied decades ago.
- There appear to be significant increased risks of prostate cancer associated with exposure to methyl bromide, captan, simazine and organochlorine group after adjusting to some common potential confounding factor including age, race and home pesticide usage (this is currently a yes/no variable, we are presently investigating the actual pesticides used in the home). The magnitudes of the risks represented by adjusted odds ratios vary according to different exposures and exposure evaluation methods, ranging from 1 to 4, with methyle bromide, captan and organochlorine all showing multiple significant ORs while simazine only showing significant risk for high diagnosis year exposure. We also noticed that all but the exposure to methyl bromide expressed an increasing trend in risk when comparing high level exposure to low level exposure. In fact, most low level exposure almost showed no increased risk while many high level exposures did.
- (These results differ slightly from those we found in Year 1, which suffered from small sample size, and lacked statistical significance as a result).

With respect to Aim 2, it appears that our method of conducting a case-control study of prostate cancer risk factors in California's Central Valley will likely result in:

- An unbiased sample of cases

- Sufficient DNA for multiple SNPs
- A more accurate method for assessing ambient pesticide exposure than has been previously utilized.
- (These results were provided after Year 1, we have verified them in the completed sample).
- The method of collecting control subjects using home visits needs to be made more efficient – the amount of effort required to obtain each eligible control subject greatly exceeded our expectations. One way we addressed this issue was to also sample the neighbors of selected control dwellings, and in our upcoming analyses we will determine the representativeness of all of our control approaches (by comparing pesticide exposures in respondent versus selected control dwellings).

When expanding this study to a full scale case-control study, we should:

- Obtain and process data from 2000 onwards from PUR and LUI (currently available)
- Design a follow-up process to immediately quantify DNA yield in specimens and return to the participant and ask for another specimen if the yield is below 10,000 ng

D. REPORTABLE OUTCOMES:

- The questionnaire used in this study was adapted from those used elsewhere, but will be made available online at the time of publication of our report of this project (particularly the questionnaire on residential history, which is central to the exposure analysis algorithm).
- The GRAPES software was developed during this study, and is available from the PI (Cockburn@usc.edu). Currently it is on a shared volume on our server, and is not made openly available because the documentation regarding its use is not complete. To date, the software has been used under supervision of the PI for 3 additional studies of pesticide exposure in the Central Valley.
- Manuscripts outlining the automation of the GRAPES process are in process.
- Other manuscripts currently being written include the following topics:
    - Comparison of DX address exposure and exposures using lifetime residential history in case-control data. Assess bias in considering only DX exposure, and build model of appropriate time sequence of exposure (i.e. time between exposure and DX, as opposed to age-specific exposure or total cumulative exposure). Aim is to come up with an exposure matrix that is biologically meaningful for specific pathways hypothesized. Compare mean exposures and resulting relative risks: DX-only exposure versus lifetime with known residential history:Versus age-specific exposure: Versus cumulative exposure (Age-weighted)
    - What is the effect of missing residential history data on residential history of pesticide exposure? Use case-control data to test the effect of various missing data imputation models to fill in holes:

        • Impact on lifetime versus age-specific, versus prior-to-DX specific exposures

- Also analyze impact of missing pesticide exposure data (1970-99 versus other times)

- Consider specific impacts of missing data from migrant populations (we know where the people missing pesticide exposure lived)

o Why is the dose-response with pesticide exposure non-monotonic?

- First, statistical test to show that it is non-monotonic

- Then show that it is not just a function of the cut points used

- Interaction with another confounder (varies by disease?) – versus competing risks, versus threshold effect.

E. CONCLUSION:

While this study is still ongoing, we believe we will be able to provide evidence that pesticide exposures appear to be strong risk factors for prostate cancer.

This study will ultimately be slightly limited by sample size, but it's purpose was to provide pilot data to justify a full scale case-control study of pesticide exposure in the development of prostate cancer. We believe that our preliminary results argue strongly for the need for a large-scale case-control study of the impact of pesticide exposures on prostate cancer.

If indeed pesticide exposure is associated with prostate cancer, the following should be considered:

- Ambient exposure to pesticides (i.e. exposure at residence, not occupational exposure) might explain increased risk of prostate cancer in certain geographical groups
- The impact of exogenous hormone exposure on prostate cancer might be substantial
- More research is required to determine what mechanisms cause pesticides to increase of prostate cancers – while these are presumably related to the hormone-mimicking affects of some pesticides, the exact mechanism, and therefore a means of prevention of prostate cancer, remain unknown.

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